

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human IL-27 in ELISAs and Western blots. In sandwich immunoassays, less than 0.2% cross-reactivity with recombinant mouse IL-27, recombinant human (rh) IL-12, and rhIL-12 p40 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human IL-27 (R&D Systems, Catalog # 2526-IL) Arg21-Lys229 (IL-27 EBI-3 subunit), Phe29-Pro243 (IL-27 p28 subunit) Accession # Q14213 (EBI-3 subunit), AAM34498 (p28 subunit)
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Human IL-27 Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 µg/mL	Human IL-27 Antibody (Catalog # AF2526)
ELISA Detection	0.1-0.4 µg/mL	Human IL-27 Biotinylated Antibody (Catalog # BAF2526)
Standard		Recombinant Human IL-27 (Catalog # 2526-IL)
Neutralization	Measured by its ability to neutralize IL-27 inhibition of EMCV-induced cytopathy in the HepG2 human hepatocellular carcinoma cell line. Bender, H. <i>et al.</i> (2009) <i>Hepatology</i> 50:585. The Neutralization Dose (ND ₅₀) is typically 1-5 µg/mL in the presence of 25 ng/mL Recombinant Human IL-27.	

DATA

Neutralization

IL-27 Inhibition of EMCV-induced Cytopathy and Neutralization by Human IL-27 Antibody. Recombinant Human IL-27 (Catalog # 2526-IL) reduces the Encephalomyocarditis Virus (EMCV)-induced cytopathy in the HepG2 human hepatocellular carcinoma cell line in a dose-dependent manner (orange line). Inhibition of EMCV activity elicited by Recombinant Human IL-27 (25 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human IL-27 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2526). The ND₅₀ is typically 1-5 µg/mL.

Western Blot

Detection of Human IL-27 by Western Blot. Western blot shows lysates of MT-2 human T cell line and CHO Chinese hamster ovary cell line either mock transfected or transfected with human IL-27 p28/IL-27A. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human IL-27 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2526) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). Specific bands were detected for the IL-27 EBI3/IL-27B at approximately 32 kDa and IL-27 p28/IL-27A at approximately 28 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

IL-27 is a heterodimeric group 2 receptor ligand molecule that belongs to the IL-6/IL-12 family of long type I cytokines (1). It is composed of EBI3 (EBV-induced gene 3), also known as IL27B, a 34 kDa glycoprotein that is related to the p40 subunit of IL-12 and IL-23, and p28, also known as IL27A, the 28 kDa glycoprotein that is related to the p35 chain of IL-12 (2-4). The human EBI3 gene encodes a 229 amino acid (aa) precursor that contains a 20 aa signal peptide and 209 aa mature protein (5). The mature region contains two potential N-linked glycosylation sites, two fibronectin type III domains, and two pairs of conserved cysteine residues with a WSXWS-like motif that places the molecule in the hematopoietin receptor family (5). Although p40, the EBI3 counterpart in IL-12, is known to form homodimers, there is no evidence to date that EBI3 also homodimerizes. Human EBI3 is 61% aa identical to mouse EBI3. The human p28 gene encodes a 243 aa precursor that contains a 28 aa signal sequence and 215 aa mature region (6). The mature region is characterized by the presence of four α -helices, placing it in the IL-6 family of helical cytokines. Human p28 is 74% aa identical to mouse p28. IL-27 is expressed by monocytes, endothelial cells and dendritic cells (7). IL-27 binds to and signals through a heterodimeric receptor complex composed of WSX-1 (TCCR) and gp130. Evidence suggests IL-27 interacts only with WSX-1 (6, 8, 9). IL-27 has both anti- and proinflammatory properties. As an anti-inflammatory, IL-27 seems to induce a general negative feedback program that limits T and NK-T cell activity (3, 7). At the onset of infection, IL-27 induces an IL-12 receptor on naïve CD4⁺ T cells, making them susceptible to subsequent IL-12 activity (and possible Th1 development) (10).

References:

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6. Pflanz, S. *et al.* (2002) *Immunity* **16**:779.
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